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(71) Applicant *(for all designated States except US)*: **CHIRON CORPORATION** [US/US]; 4560 Horton Street, Emeryville, CA 94608-2916 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **O'HAGAN, Derek** [GB/US]; Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662 (US). **VALIANTE, Nicholas** [US/US]; Chiron Corporation, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(74) Agents: **HARBIN, Alisa, A.** et al.; Chiron Corporation, Intellectual Property - R338, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

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(54) Title: ADJUVANTED MENINGOCOCCUS COMPOSITIONS

(57) Abstract: A combination of CpG oligonucleotides and polymer microparticles is an extremely effective adjuvant for Neisserial antigens. The invention therefore provides a composition comprising: (a) a Neisserial antigen; (b) a CpG oligonucleotide; and (c) a biodegradable polymer microparticle.

ADJUVANTED MENINGOCOCCUS COMPOSITIONS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

The invention relates to vaccines, more particularly those against *Neisseria meningitidis*.

BACKGROUND ART

Genome sequences for *Neisseria meningitidis* (meningococcus) serogroups A [1] and B [2,3] have been reported. The serogroup B sequence has been studied to identify vaccine antigens [*e.g.* refs. 4 to 9] and candidate antigens have been manipulated to improve heterologous expression [refs. 10 to 12].

Antigens generally require the co-administration of adjuvants in order to enhance their immunogenicity in vaccines [13]. Freund's adjuvant has been used for serogroup B meningococcus [9], and the licensed vaccine Menjugate™ against serogroup C uses aluminium hydroxide [14]. Enhancement of the bactericidal activity of *Neisseria* antigens has also been reported by using oligonucleotide adjuvants containing CpG motifs [15].

It is an object of the invention to provide further and improved adjuvants for Neisserial antigens.

DISCLOSURE OF THE INVENTION

It has been found that a combination of CpG oligonucleotides and polymer microparticles is an extremely effective adjuvant for Neisserial antigens, with the combination giving much better results than either of the individual components. The invention therefore provides a composition comprising: (a) a Neisserial antigen; (b) a CpG oligonucleotide; and (c) a biodegradable polymer microparticle.

The Neisserial antigen

The Neisserial antigen may be a protein antigen, nucleic acid encoding a protein antigen, or a saccharide antigen. The antigen preferably elicits a bactericidal or protective immune response (*e.g.* antibody response) in a recipient mammal.

The antigen may be derived from any species of *Neisseria* including *N.gonorrhoeae, N.lactamica* and *N.meningitidis*. It is preferably a *N.meningitidis* antigen and may be from any serogroup. Where the antigen is from serogroup B, it is preferred to use a protein antigen; where it is from serogroup A, C, W135 or Y then it is preferred to use a saccharide antigen. Where saccharide antigens are used, these will typically be derived from the bacterial capsular polysaccharide (*e.g.* oligosaccharides, such as those obtained by hydrolysis), and they will typically be conjugated to carrier proteins (*e.g.* to CRM_{197}).

Preferred protein antigens derived from serogroup B *N.meningitidis* are:

- a protein disclosed in any one of references 4, 5, 6, 7, 8 or 9 (in particular the 446 even SEQ IDs (*i.e.* 2, 4, 6, ... , 890, 892) disclosed in reference 4, the 45 even SEQ IDs (*i.e.* 2, 4, 6, ... , 88, 90) disclosed in reference 5 and the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241, disclosed in reference 6);
- a protein comprising an immunogenic fragment of one or more of the proteins disclosed in any one of references 4, 5, 6, 7, 8 or 9.
- a protein comprising a sequence having sequence identity (preferably greater than 50% *e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one or more of the proteins disclosed in any one of references 4, 5, 6, 7, 8 or 9.
- a protein disclosed in any one of references 10, 11 or 12.
- a protein comprising a sequence having sequence identity (preferably greater than 50% *e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to one or more of the proteins disclosed in any one of references 10, 11 or 12.

A particularly preferred protein antigen from serogroup B *N.meningitidis* is protein '287'. This protein may be used in a wild-type form [*e.g.* GenBank accession gi:7228690; alignments of polymorphic forms of 287 are shown in figures 5 & 15 of ref. 8] but derivatives of the wild-type protein may be used. For instance, proteins having 50% or more sequence identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to gi:7228690 may be used. Proteins comprising truncation or deletion variants of the protein may be used, such as the N-terminal truncated forms disclosed in references 10 to 12 ('ΔG287' in particular, in which the N-terminus of the protein up to and including the six repeated glycine residues is deleted). Fusion proteins comprising such 287 sequences may be used. All of these forms of 287, and more particularly those which retain the immunogenicity of wild-type 287 proteins, fall within the meaning of '287' as used herein.

Another particularly preferred protein antigen from serogroup B *N.meningitidis* is protein '961', also known as 'NadA' [16]. This protein may be used in a wild-type form [*e.g.* GenBank accession gi:7227256; alleles of 961 are disclosed in ref. 17] but derivatives of the wild-type protein may be used. For instance, proteins having 50% or more sequence identity (*e.g.* 60%, 70%, 80%, 90%, 95%, 99% or more) to gi:7227256 may be used. Proteins comprising truncation or deletion variants of the protein may be used, such as those disclosed in references 10 to 12 ('961c' in particular, which lacks the C-terminal membrane anchor). Fusion proteins comprising such 961 sequences may be used. All of these forms of 961, and particularly those which retain the immunogenicity of wild-type 961 proteins, fall within the meaning of '961' or 'NadA' as used herein.

Other preferred protein antigens are protein '741' and protein 'ORF46.1', and proteins 'ORF1', 'ORF4', 'ORF25', 'ORF40', 'ORF83', 'NMB1343', '230', '233', '292', '594', '687',

'736', '907', '919', '936', '953', and '983'. Other preferred protein antigens are the hybrid proteins disclosed in references 10 to 12, particularly those comprising one or more of: a 287 protein, a 953 protein, a 936 protein and/or a 741 protein.

Protein antigens may be derived from any strain of *N.meningitidis*. It is preferred to use antigens from strains 2996, MC58, 95N477 and 394/98.

As well as strain variants, single or multiple conservative amino acid substitutions may be made with altering the immunogenicity of antigens used according to the present invention.

In addition to or in place of protein antigens, nucleic acid encoding a protein antigen may be included within compositions of the invention. The nucleic acid will be expressed *in vivo* once administered to a mammalian recipient and the protein antigen will be produced. Such nucleic acid immunization is well known [*e.g.* refs. 18 to 23 *etc.*]. The nucleic acid will typically be a DNA plasmid.

A preferred saccharide antigen derived from serogroup C *N.meningitidis* is the oligosaccharide conjugate used in Menjugate™ [24, 25], which contains 12 to 22 monosaccharide units from the serogroup C capsular polysaccharide.

A preferred saccharide antigen derived from serogroup A is an oligosaccharide in which one or more of the hydroxyl groups on the constituent monosaccharide units has been replaced by a blocking group [26].

Further oligosaccharide antigens from serogroups A, W135 and Y are disclosed in reference 27.

The composition of the invention may comprise more than one Neisserial antigen. Where saccharides from both serogroups A and C of *N.meningitidis* are included, it is preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher).

The composition of the invention is preferably an immunogenic composition or vaccine. Such compositions comprise an immunologically effective amount of the antigen. By 'immunologically effective amount', it is meant that the administration to an individual of a composition of the invention comprising that amount of antigen (either in a single dose or as part of a series) is effective for raising a therapeutic or prophylactic immune response. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating physician's assessment of the medical situation, and other relevant factors. The amount may fall in a relatively broad range that can be determined through routine trials. Antigens will typically be present at a concentration of at least 1μg/ml each.

Dosage treatment may be a single dose or a multiple dose schedule (*e.g.* including booster doses).

The CpG oligonucleotide

CpG oligonucleotides are known for use as vaccine adjuvants [*e.g.* ref. 28] and they induce strong Th1 immune responses. They are useful as parenteral and mucosal adjuvants [29].

The CpG oligonucleotide used according to the present invention is a nucleic acid which includes at least one CG dinucleotide *i.e.* a cytosine nucleotide followed by a guanosine nucleotide. The oligonucleotide may contain multiple CG dinucleotides.

A CG sequence in the oligonucleotide may be flanked by two purines at the 5' side and two pyrimidines at the 3' side *i.e.* RRCGYY.

Cytosine nucleotides in the CpG oligonucleotide may be methylated, but it is preferred that they should be unmethylated.

The cytosine and guanosine nucleotides are preferably deoxynucleotides and the nucleic acid is preferably DNA. In order to enhance nuclease resistance, the oligonucleotide may comprise a modified backbone, such as a phosphorothioate backbone. As an alternative to using DNA, it is possible to use PNA (peptide nucleic acid). In addition, the oligonucleotides can comprise substitutions of the sugar moieties and nitrogenous base moieties.

The oligonucleotide preferably comprises between about 6 and about 100 nucleotides, more preferably between about 8 and about 50 nucleotides, most preferably between about 10 and about 40 nucleotides.

Oligonucleotides comprising at least one CG dinucleotide can conveniently be prepared using conventional oligonucleotide synthesis.

Examples of CpG oligonucleotide adjuvants are found in references 30 to 55.

The biodegradable polymer microparticle

Biodegradable polymer microparticles are known for use as vaccine adjuvants [*e.g.* ref. 56]. They are useful as parenteral and mucosal adjuvants.

As well as being biodegradable, the polymer used to make the microparticles will generally be sterilizable and non-toxic (biocompatible). Suitable biodegradable polymers are readily commercially available and include those derived from polyhydroxybutyric acid; polycaprolactone; polyorthoester; polyanhydride; poly(hydoxybutyrate); and a poly(α-hydroxy acid). Preferred polymers are formed from one or more poly(α-hydroxy acid) *e.g.* poly(L-lactide), poly(D,L-lactide), copolymers of D,L-lactide and glycolide (such as poly(D,L-lactide-co-glycolide), or a copolymer of D,L-lactide and caprolactone. Microparticles formed from poly(D,L-lactide-co-glycolide) ('PLG') are preferred.

These polymers are available in a variety of molecular weights, and the appropriate molecular weight for a given antigen can readily be determined. For poly(L-lactide), a suitable molecular weight will be on the order of about 2000 to 250,000. For PLG, suitable molecular weights will generally range from about 10,000 to about 200,000, preferably about 15,000 to about 150,000, and most preferably about 50,000 to about 100,000.

For PLG microparticles, a variety of lactide:glycolide ratios may be used and the ratio is largely a matter of choice, depending in part on the co-administered antigen and the rate of degradation desired. For example, a 50:50 PLG polymer, containing 50% D,L-lactide and 50% glycolide, will provide a fast resorbing copolymer while 75:25 PLG degrades more slowly, and 85:15 and 90:10, even more slowly, due to the increased lactide component. A suitable ratio of lactide:glycolide is easily determined based on the nature of the antigen and disorder in question. Moreover, mixtures of microparticles with varying lactide:glycolide ratios will find use in the formulations in order to achieve the desired release kinetics for a given antigen and to provide for both a primary and secondary immune response. Degradation rate of the microparticles of the present invention can also be controlled by such factors as polymer molecular weight and polymer crystallinity.

The term 'microparticle' as used herein, refers to a particle of about 100 nm to about 150 μm in diameter, more preferably about 200 nm to about 30 μm in diameter, and most preferably about 500 nm to about 10 μm in diameter. Preferably, the microparticle will be of a diameter that permits parenteral administration without occluding needles and capillaries. Microparticle size is readily determined by techniques well known in the art, such as photon correlation spectroscopy, laser diffractometry and/or scanning electron microscopy. The term 'microparticle' includes 'nanoparticles' [57] within its scope. Preferred microparticles are microspheres, although lamellar particles [58] may also be used.

Microparticles may be prepared using any of several methods well known in the art [*e.g.* ref. 59]. For example, double emulsion/solvent evaporation techniques [*e.g.* refs. 60 & 61] can be used to form the microparticles. These techniques involve the formation of a primary emulsion consisting of droplets of polymer solution containing the antigen (if antigen is to be entrapped in the microparticle), which is subsequently mixed with a continuous aqueous phase containing a particle stabilizer/surfactant.

More particularly, a water-in-oil-in-water (w/o/w) solvent evaporation system can be used to form the microparticles, as described in references 62, 63 and 64. In this technique, the particular polymer is combined with an organic solvent, such as ethyl acetate, dimethylchloride (also called methylene chloride and dichloromethane), acetonitrile, acetone, chloroform, and the like. The polymer will be provided in about a 2-15% solution, in organic solvent. An approximately equal amount of an antigen solution (*e.g.* in water) is added and the polymer/antigen solution emulsified

using *e.g.* a homogenizer. The emulsion is then combined with a larger volume of an aqueous solution of an emulsion stabilizer such as polyvinyl alcohol (PVA) or polyvinyl pyrrolidone. The emulsion stabilizer is typically provided in about a 2-15% solution, more typically about a 4-10% solution. The mixture is then homogenized to produce a stable w/o/w double emulsion. Organic solvents are then evaporated.

The formulation parameters can be manipulated to allow the preparation of small (<5μm) and large (>30μm) microparticles [*e.g.* 63, 65]. For example, reduced agitation results in larger microparticles, as does an increase in internal phase volume. Small particles are produced by low aqueous phase volumes with high concentrations of PVA.

Microparticles can also be formed using spray-drying and coacervation [*e.g.* refs. 66, 67 & 68]; air-suspension coating techniques, such as pan coating and Wurster coating [69, 70]; ionic gelation [71].

Prior to use of the microparticles, antigen content is generally determined so that an appropriate amount of the microparticles may be delivered to the subject in order to elicit an adequate immune response.

Antigen content of the microparticles can be determined according to methods known in the art, such as by disrupting the microparticles and extracting entrapped antigen. For example, microparticles can be dissolved in dimethylchloride and the protein extracted into distilled water [*e.g.* refs. 72, 73, 74]. Alternatively, microparticles can be dispersed in 0.1 M NaOH containing 5% (w/v) SDS. The sample is agitated, centrifuged and the supernatant assayed for antigen using an appropriate assay [75].

Antigen and/or CpG-oligonucleotides can be located within or on the microparticles. Entrapment will generally be achieved by having the antigen/oligonucleotide present during formation of the microparticles, whereas surface adsorption is achieved by adding antigen/oligonucleotide to pre-formed microparticles.

One method for adsorbing antigen/oligonucleotide onto prepared microparticles is as follows. Microparticles are rehydrated and dispersed to an essentially monomeric suspension of microparticles using dialyzable anionic or cationic detergents. Useful detergents include, but are not limited to, any of the various N-methylglucamides (known as MEGAs), such as heptanoyl-N-methylglucamide (MEGA-7), octanoyl-N-methylglucamide (MEGA-8), nonanoyl-N-methylglucamide (MEGA-9), and decanoyl-N-methyl-glucamide (MEGA-10); cholic acid; sodium cholate; deoxycholic acid; sodium deoxycholate; taurocholic acid; sodium taurocholate; taurodeoxycholic acid; sodium taurodeoxycholate; 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate (CHAPS); N-octylglucoside; 3-[(3-cholamidopropyl) dimethylammonio]-2-hydroxy-1-propane-sulfonate (CHAPSO); N-dodecyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (ZWITTERGENT 3-12); N,N-bis-(3-D-gluconeamidopropyl)-deoxycholamide (DEOXY-

BIGCHAP); sucrose monolaurate; glycocholic acid/sodium glycocholate; laurosarcosine (sodium salt); glycodeoxycholic acid/sodium glycodeoxycholate; sodium dodceyl sulfate (SDS); and hexadecyltrimethylammonium bromide (CTAB); dodecyltrimethylammonium bromide; hexadecyltrimethyl-ammonium bromide; tetradecyltrimethylammonium bromide; benzyl dimethyldodecylammonium bromide; benzyl dimethyl-hexadecylammonium chloride; benzyl dimethyltetra-decylammonium bromide. The above detergents are commercially available. Various cationic lipids known in the art can also be used as detergents [76, 77].

The microparticle/detergent mixture is then physically ground *e.g.* using a ceramic mortar and pestle, until a smooth slurry is formed. An appropriate aqueous buffer, such as phosphate buffered saline (PBS) or Tris buffered saline, is then added and the resulting mixture sonicated or homogenized until the microparticles are fully suspended. The antigen/oligonucleotide is then added to the microparticle suspension and the system dialyzed to remove detergent. The polymer microparticles and detergent system are preferably chosen such that the antigen/oligonucleotide will adsorb to the microparticle surface while still maintaining activity . The resulting microparticles containing surface adsorbed antigen/oligonucleotide may be washed free of unbound antigen/oligonucleotide and stored as a suspension in an appropriate buffer formulation, or lyophilized with the appropriate excipients, as described further below.

The antigen/CpG/microparticle combination

Various physical relationships are possible between the three basic components of the compositions of the invention. These arise because the microparticles have an internal volume and a surface, either of which may be used to locate the CpG-oligonucleotide and/or the antigen.

Thus the antigen may be entrapped within microparticles, it may be adsorbed to microparticles, or it may be in simple admixture with the microparticles without entrapment or adsorption. Adsorption is preferred.

Similarly, the CpG-oligonucleotide may be entrapped within microparticles, it may be adsorbed to microparticles, or it may be in simple admixture with the microparticles. Adsorption can be achieved using detergents such as CTAB.

The CpG-oligonucleotide and the antigen may both have the same physical relationship to the microparticles as each other, or they may be different. Likewise the CpG-oligonucleotide and the antigen may be adsorbed onto the same microparticle or the CpG-oligonucleotide and the antigen may be adsorbed onto different microparticles. All possible combinations are encompassed within the present invention:

		CpG-oligonucleotide		
		Entrapped	Adsorbed	Mixed
Anti	Entrapped	**Yes**	**Yes**	**Yes**
	Adsorbed	**Yes**	**Yes**	**Yes**

	Mixed	Yes	Yes	Yes

Compositions of the invention may include mixtures of the above *e.g.* some microparticles within the composition have entrapped antigen and some have adsorbed antigen.

Pharmaceutical compositions

For pharmaceutical use, compositions of the invention will generally comprise a pharmaceutically acceptable carrier. This gives a pharmaceutical composition of the invention.

A pharmaceutically acceptable carrier can be any substance that does not itself induce the production of antibodies harmful to the patient receiving the composition, and which can be administered without undue toxicity. Suitable carriers can be large, slowly-metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Pharmaceutically acceptable carriers can include liquids such as water, saline, glycerol and ethanol. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can also be present in such vehicles. Liposomes are suitable carriers. A thorough discussion of pharmaceutical carriers is available in ref. 78.

Compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition be prepared for oral administration *e.g.* as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops, as a spray, or as a powder [*e.g.* 79].

The pharmaceutical composition is preferably sterile. It is preferably pyrogen-free. It is preferably buffered *e.g.* at between pH 6 and pH 8, generally around pH 7.

The pharmaceutical composition may be lyophilised.

The invention also provides a delivery device containing a pharmaceutical composition of the invention. The device may be, for example, a syringe.

Medical treatments and uses

Compositions of the invention may be used therapeutically (*i.e.* to treat an existing Neisserial infection) or prophylactically (*i.e.* to prevent future Neisserial infection).

The invention provides a composition of the invention for use as a medicament.

The invention also provides a method for raising an antibody response in a mammal, comprising administering a pharmaceutical composition of the invention to the mammal. The antibody response is preferably an IgA or IgG response and it is preferably bactericidal.

The invention also provides a method for treating a mammal suffering from a Neisserial infection and/or disease, comprising administering to the patient a pharmaceutical composition of the invention.

The invention also provides a method for protecting a mammal against a Neisserial infection and/or disease, comprising administering to the mammal a pharmaceutical composition of the invention.

The invention also provides the use of (a) a Neisserial antigen, (b) a CpG oligonucleotide, and (c) a biodegradable polymer microparticle, in the manufacture of a medicament for preventing or treating disease and/or infection in an mammal.

The mammal is preferably a human. The human may be an adult or, preferably, a child. Compositions of the invention are particularly useful for immunising children and teenagers.

The uses and methods of the invention are particularly useful for treating/protecting against infections of *N.meningitidis*. The uses and methods are particularly useful for preventing/treating diseases including bacterial meningitis.

Efficacy of therapeutic treatment can be tested by monitoring Neisserial infection after administration of the composition of the invention. Efficacy of prophylactic treatment can be tested by monitoring anti-*Neisseria* immune responses after administration of the composition.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral, vaginal, topical, transdermal, ocular, nasal, aural, or pulmonary administration. Injection or intranasal administration is preferred.

Dosage treatment can be a single dose schedule or a multiple dose schedule.

Further components

Compositions of the invention may include adjuvants in addition to CpG-oligonucleotides and polymer microparticles. Preferred further adjuvants include, but are not limited to: (A) aluminium compounds (*e.g.* aluminium hydroxide, aluminium phosphate, aluminium hydroxyphosphate, oxyhydroxide, orthophosphate, sulphate *etc.* [*e.g.* see chapters 8 & 9 of ref. 13]), or mixtures of different aluminium compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous *etc.*), and with adsorption being preferred; (B) MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer) [see Chapter 10 of 13; see also ref. 80]; (C) liposomes [see Chapters 13 and 14 of ref. 13]; (D) ISCOMs

[see Chapter 23 of ref. 13], which may be devoid of additional detergent [81]; (E) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion [see Chapter 12 of ref. 13]; (F) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (G) saponin adjuvants, such as QuilA or QS21 [see Chapter 22 of ref. 13], also known as Stimulon™ [82]; (H) chitosan [*e.g.* 83]; (I) complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA); (J) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, tumor necrosis factor, *etc.* [see Chapters 27 & 28 of ref. 13]; (K) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) [*e.g.* chapter 21 of ref. 13]; (L) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [84]; (M) a polyoxyethylene ether or a polyoxyethylene ester [85]; (N) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol [86] or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol [87]; (N) a particle of metal salt [88]; (O) a saponin and an oil-in-water emulsion [89]; (P) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [90]; (Q) *E.coli* heat-labile enterotoxin ("LT"), or detoxified mutants thereof, such as the K63 or R72 mutants [*e.g.* Chapter 5 of ref. 91]; (R) cholera toxin ("CT"), or detoxified mutants thereof [*e.g.* Chapter 5 of ref. 91]; (S) double-stranded RNA and (T) other substances that act as immunostimulating agents to enhance the effectiveness of the composition [*e.g.* see Chapter 7 of ref. 13]. Alum (especially aluminium phosphate and/or hydroxide) and MF59 are preferred further adjuvants for parenteral immunisation. Mutant toxins are preferred mucosal adjuvants.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

As well as Neisserial antigen(s), the composition may comprise further antigenic components. Antigens which can be included in the composition of the invention include:

- antigens from *Helicobacter pylori* such as CagA [92 to 95], VacA [96, 97], NAP [98, 99, 100], HopX [*e.g.* 101], HopY [*e.g.* 101] and/or urease.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 102, 103, 104, 105 *etc.*
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 106, 107, 108].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 109, 110].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 110, 111].
- an antigen from hepatitis C virus [*e.g.* 112].

- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 113 & 114].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 115] *e.g.* the CRM_{197} mutant [*e.g.* 116].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 115].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 23].
- an antigen from *Chlamydia pneumoniae* [*e.g.* 117, 118, 119, 120, 121, 122, 123].
- an antigen from *Chlamydia trachomatis* [*e.g.* 124].
- an antigen from *Porphyromonas gingivalis* [*e.g.* 125].
- polio antigen(s) [*e.g.* 126, 127] such as IPV or OPV.
- rabies antigen(s) [*e.g.* 128] such as lyophilised inactivated virus [*e.g.*129, RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 115].
- antigen(s) from influenza virus [*e.g.* chapter 19 of ref. 115], such as the haemagglutinin and/or neuraminidase surface proteins
- antigen(s) from a paarmyxovirus such as respiratory syncytial virus (RSV [130, 131]) and/or parainfluenza virus (PIV3 [132]).
- an antigen from *Moraxella catarrhalis* [*e.g.* 133].
- an antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 134, 135].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 135, 136, 137].
- an antigen from *Staphylococcus aureus* [*e.g.* 138].
- an antigen from *Bacillus anthracis* [*e.g.* 139, 140, 141].
- an antigen from a virus in the flaviviridae family (genus flavivirus), such as from yellow fever virus, Japanese encephalitis virus, four serotypes of Dengue viruses, tick-borne encephalitis virus, West Nile virus.
- a pestivirus antigen, such as from classical porcine fever virus, bovine viral diarrhoea virus, and/or border disease virus.
- a parvovirus antigen *e.g.* from parvovirus B19.
- a prion protein (*e.g.* the CJD prion protein)
- an amyloid protein, such as a beta peptide [142]
- a cancer antigen, such as those listed in Table 1 of ref. 143 or in tables 3 & 4 of ref. 144.

The composition may comprise one or more of these further antigens.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [114]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred

also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens are preferably adsorbed to an aluminium salt.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using proteins antigens in the composition of the invention, nucleic acid encoding the antigen may be used. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 145. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 146.

MODES FOR CARRYING OUT THE INVENTION

Parenteral prime & mucosal boost with* Neisseria meningitidis *serogroup B antigen

Reference 6 discloses a protein from serogroup B *N.meningitidis* called '287'. References 10 to 12 disclose ways of improving its expression. One way involves deleting the N-terminus of the protein up to and including the six repeated glycine residues. This protein is referred to as 'ΔG287'.

Mice were primed and boosted with MenB ΔG287 antigen (20μg/dose) from strain 2996, formulated for intramuscular (IM) administration by adsorption to PLG microparticles, with or without CpG oligonucleotide (also adsorbed to the microparticles). An additional formulation for intranasal (IN) administration used LT-K63 adjuvant. Mice received either 3 IM doses or 2 IM then 2 IN doses (doses at: day 0; day 28; day 84; and, optionally, day 98).

Group	Formulation	Route	Dose	Antibody GMT 2 weeks after		
				dose 2	Dose 3	dose 4
1	PLG/287	IM	1, 2, 3	10,729	2,853	-
2	PLG/287 + PLG/CpG	IM	1, 2, 3	15,673	4,163	-
3	PLG/287	IM	1, 2	9,064	7,948	9,412

	287 + LT-K63	IN	3, 4			
4	PLG/287 + PLG/CpG	IM	1, 2	34,891	15,167	16,556
	287 + LT-K63	IN	3, 4			

Thus the inclusion of CpG-oligonucleotide enhanced antibody titers against intramuscularly administered MenB protein 287 (compare groups 1 & 2). Titers could be enhanced by replacing a third intramuscular dose with two intranasal doses (compare groups 1 & 3). The CpG enhancement was also seen in the intramuscular/intranasal regime (compare groups 3 & 4).

Comparison of adjuvants for MenB protein 287

ΔG287 was formulated with various adjuvants and administered to mice. Sera from the mice were assessed using the bactericidal antibody (BCA) assay and titers were as follows:

Adjuvant	BCA post-2	BCA post-3
Freund's adjuvant	2048	8192
Alum	<4	256
Alum + CpG oligonucleotide	256	4096
MF59	<4	<4
CpG oligonucleotide	<4	128
PLG microparticles (adsorbed)	8	1024
PLG microparticles (adsorbed) + CpG	2048	16384

CpG-oligonucleotide was thus only mildly effective as an adjuvant, almost comparable to alum. The PLG microparticles were more effective than both alum and CpG, but not as effective as Freund's adjuvant. In marked contrast, however, the mixture of CpG and PLG matched the adjuvanticity of Freund's adjuvant at the post-second immunization stage and exceeded Freund's adjuvant post-third immunization.

Enhancement of PLG adjuvanticity by using CpG was also seen in a separate study (02-0279):

Adjuvant	GMT post-2	GMT post-3
MF59	6967	13417
PLG microparticles (adsorbed)	7070	11367
PLG microparticles (adsorbed) + CpG	15099	26833

Effect of adsorption on adjuvanticity

The effect of adsorption on adjuvanticity was studied. Protein ΔG287 was either adsorbed onto PLG microparticles using DSS surfactant or SDS or was simply mixed with the particles. Immunisations were performed on days 0, 21 and 35 and titers were assessed on days 35 and 49. Results were as follows:

Formulation	BCA	Antibody titer 2 weeks after	
		dose 2	Dose 3
CpG + 287 adsorbed on PLG (DSS)	4096	45817	67921
CpG + 287 adsorbed on PLG (SDS)	4096	39730	29911
CpG + 287 + PLG (no adsorption)	<16	62	1065
DSS + 287 adsorbed on alum	<16	1209	1249
CpG + 287 adsorbed on Alum	1024	4054	12236
287 adsorbed on alum	128	646	2454

The adjuvanticity of CpG and microparticle mixtures for ΔG287 is thus optimal when the antigen is adsorbed to microparticles.

Reference 6 discloses a protein from serogroup B *N.meningitidis* called '961' (now known as 'NadA' [16,17]). References 10 to 12 disclose ways of improving the expression of NadA. One way involves deleting the C-terminus of the protein to remove its membrane anchor (*i.e.* remove amino acids 351-405 for strain 2996), as well as natural removal of its leader peptide. This protein is referred to as '961c'. The effect of adsorption on PLG adjuvanticity when co-administered with CpG was studied for 961c, as described above for 287:

Formulation	BCA	Antibody titer 2 weeks after dose 3
961 adsorbed on PLG (SDS)	2048	20661
961 + PLG (no adsorption)	256	1706
287 adsorbed on PLG	4096	63057
287 adsorbed on PLG + 961 soluble	4096	287: 86052; 961: 1924
287 adsorbed on PLG + 961 adsorbed on PLG	8192	287: 107142; 961: 11717
287 (not adsorbed) + 961 (not adsorbed) + 'blank' PLG	1024	287: 1266; 961: 145
287 (adsorbed) + 961 (adsorbed) + 'blank' PLG	8192	287: 78176; 961: 20876

As for ΔG287, therefore, the adjuvanticity of CpG and microparticle mixtures for 961c is optimal when the antigen is adsorbed to microparticles. This is true for the antigen on its own and the antigen when combined with ΔG287.

For both ΔG287 and 961c, therefore, singly and combined, the best adjuvanticity for CpG and PLG mixtures is seen when the antigens are adsorbed onto the PLG microparticles.

PLG, CpG, alum and MF59

Various combinations of PLG, CpG and alum were tested for protein ΔG287, expressed as a His-tagged product. Serum bactericidal titers after three immunisations were as follows:

Adjuvant	Titer
Alum	2048
Alum + CpG	32768
MF59	8192
MF59 + CpG	32768
PLG (antigen adsorbed to PLG)	1024
PLG + CpG (antigen and CpG both adsorbed to PLG)	4096
PLG + MF59 (antigen adsorbed to PLG)	2048
PLG + MF59 + CpG (antigen adsorbed to PLG)	8192
Complete Freund's	32768
PLG + Complete Freund's (antigen adsorbed to PLG)	2048

Similar experiments were performed and results were as follows:

Adjuvant	Titer
PLG (antigen adsorbed to PLG)	1024
PLG + CpG (antigen adsorbed to PLG)	16384
PLG + CpG (antigen and CpG both adsorbed to PLG)	16384
PLG + alum (antigen adsorbed to PLG)	1024
PLG + alum + CpG (antigen adsorbed to PLG)	16384
PLG + alum + CpG (antigen and CpG both adsorbed to PLG)	8192
PLG + MF59 (antigen adsorbed to PLG)	4096
PLG + MF59 + CpG (antigen adsorbed to PLG)	16384
Alum (antigen adsorbed to alum)	256
CpG	128
Alum + CpG	1024
Alum + CpG + PLG (antigen adsorbed to alum; CpG adsorbed to PLG)	4096
CpG + PLG (CpG adsorbed on PLG; antigen not adsorbed)	64

Thus MF59 and alum can further enhance efficacy of CpG/PLG mixtures, adsorption of CpG to PLG microparticles is not necessary for adjuvanticity, but adsorption of antigen to microparticles is again seen to be optimal.

Antigen mixtures

The effect of adsorption on adjuvanticity was studied for proteins ΔG287 and 961c, singly and in combination. Antibody titers after three doses were as follows:

Formulation	Antibody GMT against	
	287	961
CpG + 961 adsorbed on PLG	-	20661
CpG + 961 + PLG (no adsorption)	-	1706
CpG + 961 + 287 adsorbed on PLG	86052	1924
CpG + 961 adsorbed on PLG + 287 adsorbed on PLG	107142	11717
CpG + 287 adsorbed on PLG	63057	-
CpG + 287 & 961 co-adsorbed on PLG	57306	6251
CpG + 961 adsorbed on PLG + 287 adsorbed on PLG + PLG	78176	20876
287 + 961 + PLG (no adsorption of antigens)	1266	145

As for ΔG287, therefore, the adjuvanticity of CpG and microparticle mixtures for protein 961c is optimal when the antigen is adsorbed to microparticles.

Further combinations of adjuvants with PLG microparticles were tested for proteins ΔG287 and 961c. The CpG was either soluble or was adsorbed to PLG microparticles. Results were as follows:

Formulation + PLG microparticles	BCA	GMT against	
		287	961
287 (adsorbed on PLG) + 961 (adsorbed on PLG)	256	5719	2412
287 (adsorbed on PLG) + 961 (adsorbed on PLG) + CpG	512	17553	8627
287 (adsorbed on PLG) + 961 (adsorbed on PLG) + CpG (adsorbed on PLG)	1024	16906	6720
287 (adsorbed on PLG) + 961 (adsorbed on PLG) + MF59	64	4636	3969
287 (adsorbed on PLG) + 961 (adsorbed on PLG) + MF59 + CpG	2048	23642	48446

Similar work was performed on groups of 10 CD-1 mice, using 20μg per PLG-adsorbed antigen per IM dose (days 0, 21 and 35). Where CpG was present, it was given at 10μg per dose. ELISA titers (GMT) were calculated as the reciprocal serum dilution giving OD_{450nm} 0.5, and sera were tested for both antigens. Serum bactericidal activity titers (SBA) are calculated as the reciprocal serum dilution killing 50% of target bacteria, and sera were tested for activity against 2996 strain and against MC58, a heterologous strain. Titers at day 49 (2 weeks post-third dose) were as follows:

287	961	Extra adjuvant	GMT		SBA	
			287	961	2996	MC58
X	–	–	8375	–	512	<4
X	–	Soluble CpG	33736	–	1024	128
X	–	PLG-adsorbed CpG	32058	–	1024	64
–	X	–	–	3818	nd	nd
–	X	Soluble CpG	–	14149	2048	<4
–	X	PLG-adsorbed CpG	–	18526	2048	<4

X	X	–	13557	2476	nd	nd
X	X	Soluble CpG	21664	6557	8192	64
X	X	PLG-adsorbed CpG	27259	7510	2048	128
X	X	Soluble CpG + MF59	27981	26826	2048	256
Control: soluble 287 with CFA			37889	–	1024	<32
Control: soluble 961 with CFA			–	50453	4096	<4
Control: soluble 287 and 961 with CFA			1678	27069	512	<32

Reference 12 discloses a combination of three proteins which, between them, include five different *N.meningitidis* antigens: (1) $961c_{2996}$; (2) $\Delta G287_{NZ}$–953_{2996}; and (3) 936_{2996}–$\Delta G741_{MC58}$. The antigen mixture was tested in reference 12 using aluminum hydroxide adjuvant. According to the present invention, the antigen mixture is adjuvanted by adsorption to a biodegradable polymer microparticle plus a CpG oligonucleotide. Titers after the third dose were as follows:

Immunisation	ELISA GMT				SBA (against seven strains)						
	961	287	741	953	2996	MC58	BZ133	394/98	NGH38	F6124	44/76
(1) 961 on alum	12346	–	–	–	4096	<4	<4	<4	<4	64	<4
(2) 287-953 on alum	–	6415	–	585	1024	1024	256	1024	4096	256	1024
(3) 936-741 on alum	–	–	10625	–	<4	32768	16384	1024	128	16384	32768
(1), (2) & (3) on alum	42302	18206	33881	4549	8192	32768	32768	2048	4096	32768	65536
(1) 961 on PLG	14185	–	–	–	2048	4	<4	<4	16	256	<4
(2) 287-953 on PLG	–	43515	–	478	2048	128	2048	2048	8192	4096	128
(3) 936-741 on PLG	–	–	16150	–	<4	32768	16384	1024	512	8192	262144
(1), (2) & (3) on PLG	6735	24304	13801	1214	4096	65536	32768	2048	4096	32768	65536
(1), (2) & (3) on PLG + CpG	10896	40697	26966	2301	8192	262144	65536	4096	8192	32768	262144

Compared to the aluminum adjuvant used in reference 12, the PLG+CpG mixture leads to lower overall antibody titers (except for protein 287) but, importantly, gives higher bactericidal titers against a wide range of strains. Although absolute titers are lower, therefore, the adjuvant of the invention therefore advantageously shifts antibody production towards bactericidal antibodies.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. An immunogenic composition comprising: (a) a Neisserial antigen; (b) a CpG oligonucleotide; and (c) a biodegradable polymer microparticle.

2. The composition of claim 1, wherein the Neisserial antigen is a protein antigen.

3. The composition of any preceding claim, wherein the Neisserial antigen can elicit a bactericidal immune response in a recipient mammal against *Neisseria meningitidis*.

4. The composition of claim 3, wherein the Neisserial antigen comprises a *N.meningitidis* protein selected from the group consisting of: NadA protein, or a variant thereof; 287 protein, or a variant thereof; 741 protein, or a variant thereof; 953 protein, or a variant thereof; and protein, or a variant thereof.

5. The composition of any preceding claim, wherein the CpG oligonucleotide comprises between about 6 and about 100 deoxyribonucleotides.

6. The composition of any preceding claim, wherein the biodegradable polymer microparticles comprise a poly(α-hydroxy acid).

7. The composition of claim 6, wherein the microparticles comprise poly(D,L-lactide-co-glycolide).

8. The composition of any preceding claim, wherein a Neisserial antigen is entrapped within the microparticles.

9. The composition of any preceding claim, wherein a Neisserial antigen is adsorbed to the microparticles.

10. The composition of any preceding claim, wherein a CpG oligonucleotide is entrapped within the microparticles.

11. The composition of any preceding claim, wherein a CpG oligonucleotide is adsorbed to the microparticles.

12. The composition of any preceding claim, comprising a further adjuvant.

13. The composition of claim 12, comprising MF59 adjuvant.

14. The composition of claim 12, comprising an aluminum salt adjuvant.

15. The composition of any preceding claim, comprising at least one further non-Neisserial antigens.

16. The composition of any preceding claim, further comprising a pharmaceutically acceptable carrier.

17. The composition of any preceding claim, for use as a medicament.

18. A method for raising an antibody response in a mammal, comprising administering to the mammal the composition of any one of claims 1 to 16.

19. A method for treating a mammal suffering from a Neisserial infection and/or disease, comprising administering to the patient the composition of any one of claims 1 to 16.

20. A method for protecting a mammal against a Neisserial infection and/or disease, comprising administering to the patient the composition of any one of claims 1 to 16.

21. The use of (a) a Neisserial antigen, (b) a CpG oligonucleotide, and (c) a biodegradable polymer microparticle, in the manufacture of a medicament for preventing or treating disease and/or infection in an mammal.